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The 5' Terminal Region of the Apocytochrome b Transcript in *Crithidia fasciculata* is Successively Edited by Two Guide RNAs in the 3' to 5' Direction

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I analysed the chimeric gRNA-mRNA molecules in *C. fasciculata* that are predicted to transiently exist in editing of the 5' terminal domain of apocytochrome b (CYb) mRNA, by PCR amplification and DNA sequencing, and obtained evidence indicating that among the fourteen editing sites numbered from 3' to 5', one guide RNA species (gRNA-I) directs the sequence from site 1 to the first U residue at site 7 (3' block) and the other guide RNA species (gRNA-II) directs the sequence from the second U residue at site 7 to site 14 (5' block), and that the direction of editing in each block is 3' to 5'. I also found that a stretch of the edited sequence in the 3' block of mRNA can form a stable duplex with a stretch immediately upstream of the guide sequence in gRNA-II. The result leads to a successive editing model that the 3' block of pre-edited mRNA is first edited by gRNA-I, and after completion of editing, the 5' portion of gRNA-II basepairs with the edited mRNA for editing of the 5' block.

Keywords: Kinetoplastid/ Mitochondria/ gRNA-mRNA chimera molecules/ Polymerase chain reaction/ Transesterification model

Several mitochondrial mRNA in kinetoplastid protozoans such as *Crithidia*, *Leishmania* and *Trypanosoma* are extensively edited after transcription [1]. The location of editing domains, number of editing sites within a single editing domain, and number of U residues to be added or deleted at each editing site are very specific to individual mRNAs. As to the mechanism of RNA editing, a model that small RNAs, named guide RNA or gRNA, specify the sequence alternation has been proposed, based on the finding that the intergenic regions of maxicircles contain some sequences that can basepair with edited mRNA sequences if G:U pairs are allowed. This model was experimentally verified by detection of small RNA species with oligo(U)_n tails that can hybridize

to the corresponding regions of the maxicircles and minicircles, and further by identification of chimeric gRNA-mRNA molecules containing U clusters covalently linked at sites of RNA editing [2]. Nevertheless, the precise mode of action of the gRNA molecules is yet unknown. According to the computer search data, some of a single editing domain are covered by a few different gRNA species, but its molecular mechanism is also an unsettled question.

The 5' terminal region of the transcript of the apocytochrome b (CYb) cryptogene in *C. fasciculata* contains fourteen editing sites, including the one that generates the AUG initiation codon [3]. These sites are tentatively numbered in the 3' to 5' direction. By

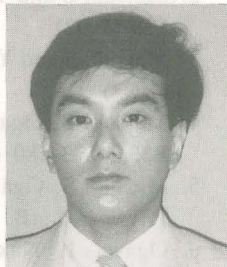
RESEARCH FACILITY OF NUCLEIC ACIDS

Scope of Research

With emphasis on regulatory mechanisms of gene expression in higher organisms, the research activity has been focused on analysis of signal structures at the regulatory regions of transcriptional initiation and of molecular mechanisms involved in post-transcriptional modification by the use of eukaryotic systems appropriate for analysis. As of December 1994, studies are concentrated on the molecular mechanism of RNA editing in mitochondria of kinetoplastids and gene expression of human retroviruses.



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computer analysis, two candidate gRNAs, named gRNA-I and gRNA-II, have been assigned for editing of sites 1 to 4 (3' or downstream block) and sites 5 to 14 (5' or upstream block), respectively. The existence of two gRNA species in cells has also been demonstrated. The editing pattern is well conserved between *L. tarentolae* and *C. fasciculata*, except that mRNA in *L. tarentolae* contains an additional editing site at the most 3' end and one less U residue at the most 5' editing site. The computer assignments of the editing blocks by gRNA-I and gRNA-II are very similar in both strains, but no direct evidence supporting these predictions has been presented.

To gain information on the mechanism how different gRNAs specify the RNA sequence alternation in a single editing domain, we analysed the kinetoplast RNA (kRNA) of *C. fasciculata* by PCR amplification and DNA sequencing, assuming that both putative gRNA-I and gRNA-II undergo transient covalent interaction with mRNA during editing process. As a result, we could identify the chimeric molecules of both gRNA-I and gRNA-II linked with partially edited mRNA.

Detection of both gRNA molecules that are covalently linked to partially edited mRNA through oligo(U)_n strongly supports the transesterification model (3). Allowing G: U pairing, the gRNA-I sequence in the chimeric molecules can fold back on the edited mRNA sequences from editing site 1 to the first U in editing site 7 and the gRNA-II sequence on the remaining mRNA sequence. According to the transesterification model, the 3' terminal U of gRNA, that formed a duplex with preedited mRNA at the anchor site, first attacks mRNA at the first mismatched base and produces the chimeric molecule by transesterification. The U stretch in the gRNA molecule then basepairs with the guide A or G residue of the gRNA itself, and the second transesterification takes place at the next mismatched base. Oligo(U)_n in the majority of clones is connected with the G residue just 3' of editing site 7, but the A residue just 5' of the oligo(U)_n can basepair with the first U within editing site 7 (see Figure 1 of Reference 5). It is therefore likely that the transition site of editing from gRNA-I to gRNA-II is between the first and second U residues within editing site 7. The mRNA moieties that were connected to oligo(U)_n in all the clones have completely been edited. Thus it is evident that the editing reaction progressively proceeds in the 3' to 5' direction in both the editing blocks. This is essentially consistent with the conclusion deduced from analysis of preedited-edited mRNA junctions.

When the two gRNA sequences were assigned on the mRNA sequence, we noted that the 12 bases long sequence of edited mRNA, 5'-uGuuAuuuAGAA-3', from editing sites 4 to 6 can basepair with both the guide sequence of gRNA-I and the 5' moiety of gRNA-II (see Figure 3 of Reference 5). The gRNA-II molecule does

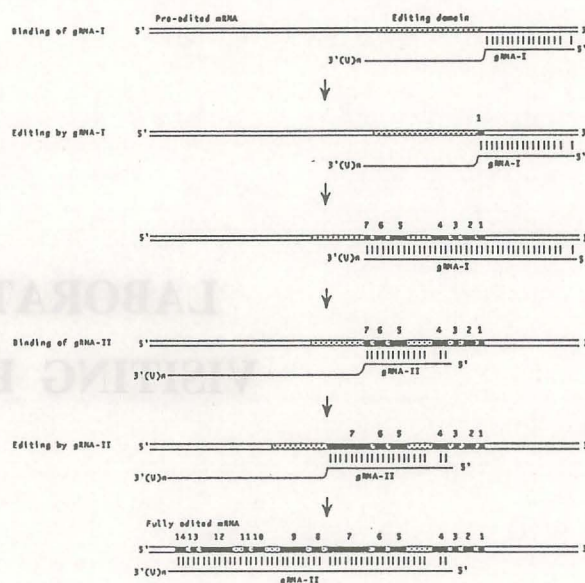


Figure 1. Diagram of a sequential editing model by two different gRNA molecules. mRNA is indicated by double lines, on which the entire editing domain is shown by hatched boxes and inserted U residues at individual editing sites by filled boxes. Single lines represent the gRNA molecules and basepairs between mRNA and gRNA are indicated by vertical lines.

not carry any region that can form a stable duplex with preedited mRNA. Although the 5' terminus of gRNA-I has not been determined yet, the region upstream from the guide sequence of gRNA-I can basepair with the mRNA region just downstream of the 3' block (see Figure 3 of Reference 5). Assuming that the regions of gRNA molecules which can form stable duplexes with mRNA provide the anchor sites for mRNA editing (see Figure 3 of Reference 5), an editing model emerged is schematically shown in Figure 1. The editing reaction on mRNA of the CYb cryptogene first initiates by basepairing-mediated recognition of the preedited mRNA sequence with the anchor sequence of gRNA-I. Followed by editing of the 3' block with the guide sequence of gRNA-I, the anchor sequence of gRNA-II recognizes the edited mRNA sequence and initiates editing of the 5' block. Although the mechanism involved in switching of binding from gRNA-I to gRNA-II is not known, the edited mRNA sequence can form a more stable duplex with the anchor sequence of gRNA-II than that with the guide sequence of gRNA-I.

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